

## Relationship between Witches' Broom Protein and Dynamic of Some Amino Acids in *Paulownia* Tree Leaves

Fan Guoqiang (范国强) Jjiang Jianqing (蒋建平)

Henan Agricultural University, Zhengzhou 450002, P. R. China

**Abstracts** This paper dealt with the SDS polyacrymide gel electrophoresis of proteins and analysis of the amino acids in the diseased and healthy leaves with the same strain respectively, which were at the same side and height, of *Paulownia catalpifolia*, *P. elongata*, *P. albiphloea* and *P. kawakamii* respectively. The results indicated that the leaves of 4 species of *Paulownia* trees with witches' broom had one protein band, of which molecular weight was 12 kD, which did not appear in the healthy leaves free of phytoplasmas. Moreover, the protein quantity in the affected leaves was more than that in the healthy leaves free of phytoplasmas; At the same time, there was significant difference on the amino acids between the healthy and diseased leaves of *P. catalpifolia* and *P. kawakamii*. The amount of cystine in the affected leaves was higher than that in the healthy leaves, but the change of amount of phenylalanine in the affected and healthy leaves was contrary. These changes of proteins and amino acids in the leaves might be related to the witches' broom of the *Paulownia* trees.

**Key words:** *Paulownia*, Witches' broom, Protein, Amino acid

### Introduction

Witches' broom is a serious disease to the growth and its life of *Paulownia* trees and is one of problems needed to be solved urgently in forestry. Ten years ago, physiological and biochemical changes during the occurrence of witches' broom were found out (Jiang, 1992; Ren, 1987). Recent years, some aspects on phytoplasmas have studied by Lin (1993) and Zhang (1994) with molecular methods, but there has not been much progress to be made yet. This paper studied the differences between the affected and healthy trees, based on the final product of gene expression in leaves of *Paulownia* trees--protein and its basic component units (amino acids). It will provide basic data for revealing the mechanism of witches' broom of *Paulownia* trees.

### Materials and Methods

Materials were the diseased and healthy leaves on the same affected tree (DL and DHL) and healthy leaves on the healthy tree (HL) collected on May 5, 1995 which were at the same side and same height of the same strain of *Paulownia catalpifolia*, *P. elongata*, *P. albiphloea* and *P. kawakamii*, from Forestry Experiment Station of Henan Agricultural University located at the suburb of Zhengzhou City. The leave protein powder were made as described as Fan (1997).

#### Protein extraction and SDS-PAGE

Protein

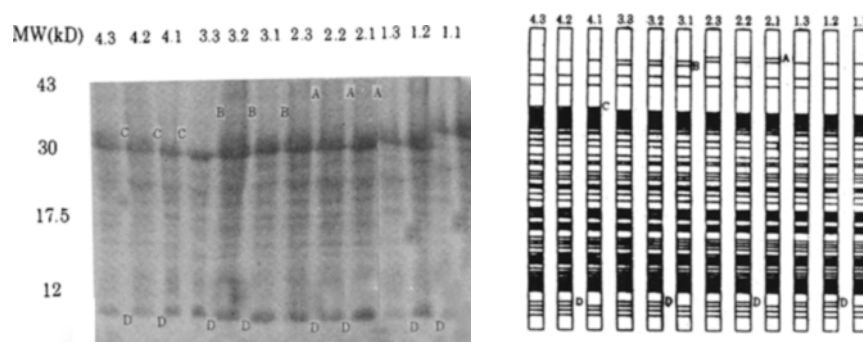
extraction and electrophoresis were processed as Laemmili (1970) and Fan (1997).

**Contents of amino acids** The DL, DHL and HL of *Paulownia catalpifolia* and *P. kawakamii* desiccated at 80 °C for 3h were grounded into fine powders and 30 mg of the powders were dehydrolysed completely with 6 mol/L HCL in sealed vacuum condition. The solution were analyzed as described in the handbook of Hitachi 835-50 amino acid analyzer to determine the contents of amino acids.

### Results and Analyses

#### SDS-PAGE of protein in DL, DHL and HL of *Paulownia* trees

The profile (Fig. 1) of SDS-PAGE of protein in DL, DHL and HL of *P. catalpifolia*, *P. elongata*, *P. albiphloea* and *P. kawakamii* shown that proteins in different *Paulownia* tree leaves have great differences in protein band (molecular weight above 43 KD). That is, *P. elongata*, *P. albiphloea* and *P. kawakamii* had one more band (A, B, C) than *P. catalpifolia* respectively, which indicated that there were gene differences among the four strains and that the protein (band D) existed in DL and DHL had not been found in HL, of which molecular weight is 12 KD, but the amount of this protein in DL was higher than that in DHL. This meant that the protein had some relationship to the witches' broom and the amount of the protein to the degree of the tree affected diseased.



**Fig. 1. SDS-PAGE of protein in different species of *Paulownia* tree leaves**

1.1, 1.2, 1.3: Healthy, diseased-healthy, healthy leaves of *P. catalpifolia*  
 2.1, 2.2, 2.3: Healthy, diseased-healthy, healthy leaves of *P. lankauensis*  
 3.1, 3.2, 3.3: Healthy, diseased-healthy, healthy leaves of *P. albiphoea*  
 4.1, 4.2, 4.3: Healthy, diseased-healthy, healthy leaves of *P. kawakamii*

### The content of amino acids in *Paulownia* tree leaves

The result of analysis of amino acids in DL, DHL and HL of *P. catalpifolia* and *P. kawakamii* indicated that the content of amino acids in the leaves affected differently of the same strain was different, which meant that the protein component in leaves had changed after the trees were affected. For *P. catalpifolia* and *P. kawakamii* trees, the change trends of cys. and phe. in the leaves were the same among the analyzed amino acids (the amount of cys. in leaves increased with the diseased worsen, but the phe. decreased) The changing trend of cys. might have some relationship to the witches' broom of the trees. The change of base amino acids (Lyr+His+Arg) in the leaves of *P. calpifolia* and *P. kawakwmii* was that the amount in DL, DHL and HL was the lower, low and higher, which related to gene expression in the leaves.

**Table 1. Content of amino acids in diseased and healthy leaves of *Paulownia* trees mg amino acids/g leaves**

	CHL	CDL	CDHL	KHL	KDL	KDHL
Asp.	14.67	23.94	20.77	26.32	24.46	23.12
Thr.	5.69	8.79	7.74	9.87	7.95	8.10
Ser.	8.98	16.66	25.00	24.01	18.35	24.00
Glu.	3.59	21.52	19.01	14.80	12.84	16.82
Pro.	16.76	24.85	25.35	28.94	24.46	22.52
Gly.	5.69	1.51	8.80	6.25	8.56	2.70
Ala.	2.99	1.21	4.58	3.62	1.56	3.30
Cys.	1.20	2.73	2.46	1.64	4.59	3.90
Vsl.	1.80	3.94	3.87	3.62	2.47	2.40
Met.	1.80	1.82	1.27	4.60	3.98	2.20
Leu.	2.69	2.76	5.28	3.32	3.32	2.70
Phe.	6.36	3.89	5.63	7.56	1.50	5.81
Lyr.	7.89	4.79	5.98	8.22	3.90	7.34
His.	2.42	1.50	1.76	2.63	1.90	2.14
Arg.	7.58	4.79	6.69	8.55	8.71	7.34

Note: The values given in the above table are means based on three repli-

cates

### Discussion

Protein is the final product of gene expression. Any protein in the plant had its own gene responding to it (Richter, 1978). When environment changed, the metabolism of plant changed also, which would cause the change of gene expression of plant. For *Paulownia* trees, the invading of pytoplasmas might cause physiological and biochemical changes. Although study of these changes was favorable to explain the relationship between the change and witches' broom, it was difficult to elucidate the mechanism of witches' broom occurring. It is commonly known that amino acids are the basic units of proteins and proteins the final products of gene expression, thus there are much significance to study the relationship between protein and witches' broom of *Paulownia*, Saho (1957) found that no difference in either the ethanol or the HCL extracted amino acids existed between healthy and diseased leaves, but the content of amino acids in small leaves of diseased twigs was 1/5 of that in the healthy ones, this dis coincided with the results presented in this paper, which might be because that amino acids determined in these two experiments were different. The results in this paper, based on the gene direct expression--protein, indicated the relationship between protein and witches' broom, which had been verified with the difference of amino acids of the protein among DL, DHL and HL of *P. catalpifolia* and *P. kawakamii*, Which would provide basic data for explanation of the mechanism of witches' broom in the future.

## References

- Fan Guoqiang. 1997. Study on the methods of extracting protein from *Paulownia* leaves. Plant bulletin.
- Laemilli UK. 1970. Cleavage of structural proteins during the assembly of the head of basleriophage T4. Nature, 227:680-685
- Lin Mulan. et al. 1993. Production and preliminary application of monoclonal antibodies against paulownia witches' broom MLO. 35 (9): 710-715
- Jiang Jianping. et al. 1993. Primary study on the relationship between witch'broom of *Paulownia* and peroxidase content. Acta Agriculturae Universitatis Henanensis.
- Ren Guolan. et al. 1987. Study on treatment of withch 'broom of *Paulownia* with qucongling. Acat Agriculturae Universitatis Henanensis. 21: (1) 96-98
- Richter G. 1978. Physiology and Biochemistry of Primary Metabolism. Florida: CRC Press. 3778-387
- Zhang Chunli. et al. 1994. Molecular colon and analysis of MLO of witch' broom of Paulownia. Acta of Botanica Sinica. 36 (4): 278-282

(Responsible Editor: Dai Fangtian)